

CLAIMS

1. A method for detecting ALK tyrosine kinase activity, which comprises the following steps:

- 5 i) incubating the ALK protein or a functional derivative thereof with a peptide substrate selected from SEQ ID N. 1 or 2 in conditions suitable for phosphorylation of the peptide;
- ii) detecting the phosphorylated peptide.

2. A method according to claim 1, wherein the peptide has sequence SEQ ID
10 N. 1.

3. A method according to claim 1, wherein purified ALK protein or an ALK-containing preparation is used.

4. A method according to claim 3, wherein said preparation is a cell lysate.

5. A method according to claim 1, wherein said functional derivative
15 contains the entire catalytic domain of ALK spanning residues 1116-1392 of ALK sequence.

6. A method according to claim 5, wherein said functional derivative is a fragment of ALK protein extending from residue Leu¹⁰⁷³ to Ala¹⁴⁵⁹.

7. A method according to claim 6, which comprises the steps of:

- 20 a) adhering a peptide of SEQ ID N. 1 or 2 to a solid phase;
- b) incubating the solid phase with said ALK fragment in conditions suitable for tyrosine phosphorylation;
- c) washing the solid phase;
- d) incubating the solid phase with an anti-phosphotyrosine antibody
25 (primary antibody) in conditions suitable for antigen-antibody binding;
- e) washing the solid phase;
- f) incubating the solid phase with an enzyme-conjugated antibody

(secondary antibody) recognizing the primary antibody in conditions suitable for the binding of primary and secondary antibodies, so that a ternary immune complex is formed;

g) washing the solid phase;

5 h) measuring the enzymatic activity of the immune complex wherein the measured activity is proportional to the amount of tyrosine-phosphorylation.

8. A method according to claim 7, wherein the enzyme conjugated to the antibody is Horse-Radish peroxidase.

10 9. A method according to claim 7, wherein the enzymatic activity is detected by colorimetric reaction.

10. A method according to any previous claims, for the identification of compounds that modulate ALK tyrosine-kinase activity.

11. A method according to claim 10, which comprises the steps of

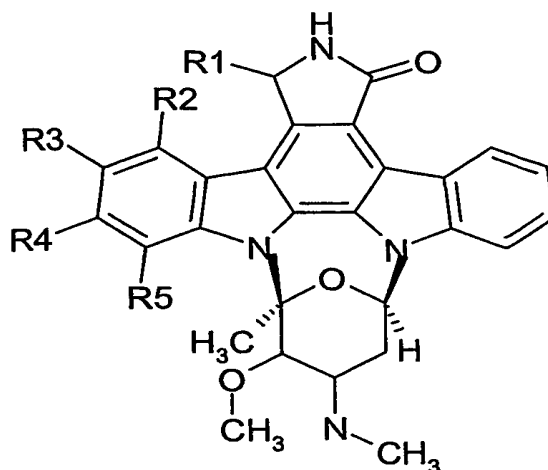
15 i) incubating ALK protein or a functional derivative thereof with a peptide selected from SEQ ID N. 1 or 2 in the presence of a candidate compound (a) in conditions suitable for peptide phosphorylation;

ii) detecting the phosphorylated peptide thus formed;

20 12. A method according to claims 10-11, wherein the ALK-modulating activity of the candidate compound is compared to that of a reference compound which is assayed under the same conditions as the candidate compound.

25 13. A method according to claim 12, wherein the reference compound is staurosporine.

14. A method according to claim 12, wherein the reference compound is a staurosporine derivative of general formula (I):



wherein R1 and R2, independently of one another, are selected from halogen, preferably chlorine, phenyl or C1-C3 alkyl optionally substituted with one or more halogens; R3 is hydroxyl; R4 is hydroxyl or hydroxymethyl; R5 is C1-C3 alkyl, optionally halo-substituted, or benzyl.

15. A peptide useful as ALK substrate selected from SEQ ID N. 1 or 2.

16. A peptide according to claim 15, which is SEQ ID N. 1.

17. The use of a peptide according to claim 15 or 16 for the determination of ALK tyrosine-kinase activity.

18. The use of a compound of formula (I), as per claim 14, for the preparation of a medicament for the treatment of ALK-related tumors, especially anaplastic large cell lymphomas and non-Hodgkin lymphomas.

19. A kit for detecting ALK tyrosine-kinase activity according to claims 1-14, which comprises a peptide of SEQ ID N: 1 or 2 and an anti-phosphotyrosine antibody.

20. A kit according to claim 19, containing an additional component selected from reagents for colorimetric reactions, buffers, diluents, detergents, stabilizers, staurosporine or a derivative thereof as per claim 14.